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Genetic Variance In Flax Cultivars Using RAPD Technique, Cluster And Principal Component Analysis

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Abstract

In the study, eight flax (Linum usitatissimum L.) genotypes were used (Sakha 1, Sakha 2, Sakha 3, Sakha 5, Sakha 6, Poloni, Geza and Syrian), which were planted in the fields of the College of Science / University of Kirkuk during the 2020-2021 season according to the RCBD design with three replications. DNA was extracted from plant leaves for the studied genotypes in the laboratory of Wahaj Aldna Co. during the year 2021 according to the attached method (Geneaid@genomic DNA mini kit plant) with the extraction kit, thirteen primers were tested and the results of using those primers in RAPD reactions showed a difference in the number of amplified sites and their molecular sizes according to the primer used, resulting from the difference in the number of sites complementing that primer in the genome of each of the flax cultivars included in this study. The thirteen primers used in this study showed All of the technology doubled in locations as it showed bundles of varying numbers and locations among the varieties under study, and produced a total of 202 bundles, which included 192 different bundles with a percentage of 95.05%, while identical bundles (main bundles) amounted to only 10 bundles with a percentage of 4.95%. The lowest value recorded for the genetic dimension among the studied cultivars was 0.365 between the two cultivars Sakha 5 and Sakha 6, while the highest genetic dimension was between the two cultivars Sakha 1 and Sakha 6 with a value of 0.582, and the results of the Principal Analysis Component (PCA) showed a clear agreement with the results of the dendrogram, The results of the distribution showed the matching of the groups that make up the dendrogram based on the RAPD indicators, which shows their distribution in three main groups, the first group included the two cultivars Sakha 5 and Sukha 6, while the second group included the two cultivars Sakha 2 and Sakha 3, while the third major group included the rest of the varieties.

Keywords: genetic variation, flax, Linum usitatissimum L. RAPD, cluster analysis, Principal Analysis Component.

Introduction

The flax crop Linum usitatissimum L., is a typical plant species for multi-purpose uses with the full use of plants for several purposes, including industrial food, animal feed, fiber, nutrients and medicine, As each part of the flax plant has a specific economic importance, its seeds have a high oil content, and due to its dyeing properties, the oil is used in the preparation of paints, varnishes, polymer industries, printing ink and many countless by-products, and the stem produces fibers with tensile strength. It is durable and has good blending property, the stem produces fibers with tensile strength, durability, and good blending property, the remaining cortical tissue serves as a source of wax and raw materials for papermaking (Akbar et al., 2003).

Diversity in germplasm is necessary to meet different purposes of the crop such as increased yield, wider adaptation, desired quality and resistance to pests and diseases (Begum et al., 2007). Information about the extent and nature of the interrelationship between traits helps in formulating an effective scheme for selecting some traits. In addition, knowledge of the naturally occurring diversity in a population helps identify diverse groups of genotypes (Tadesse et al., 2009).

Methods for measuring genetic diversity include classical genetic analysis to assess variance in single genes, multivariate analysis to analyze variance in polygenic traits, genealogical analysis and molecular analysis to detect genetic diversity using biochemical and molecular markers. Due to their large number, ease of handling, and often being dominant in nature and reliable scoring methods, molecular markers are the best tools for determining genetic relatedness in any species (Ijaz et al., 2013).

DNA fingerprinting is used to determine genetic diversity within breeding populations, to positively identify and distinguish accessions, cultivars and species that may be difficult to characterize due to similar morphological characteristics or unclear traits, and to identify plants that contain genes of interest such as the confirmation of transformation events (Saunders and Hemeida, 2001). One PCR-based DNA marker technique, random amplified DNA marker (RAPD), has been widely used to determine genetic makeup in crop plants, and has gained importance due to its simplicity, efficiency, and no requirement for sequencing information requirement (Karp et al., 1997).

The use of molecular markers, such as simple sequence repeat (SSR) and random amplified DNA polymorphism (RAPD), are a powerful tool for analyzing plant genome structure and function. Once molecular markers have been identified in multiple communities over multiple generations and in multiple environments, plant breeders can Use this data to select such positive markers to develop breeding populations with desirable traits (Gulla et al., 2021).

Cluster analysis is widely used in agriculture to process data for different crops such as rapeseed (Rameeh, 2015), barley (Fotokian et al., 2014), as well as flax (Bakry et al., 2014). Cluster analysis divides data into groups, and this is important in apportioning variance over a large number of cultivars, or to revealing genetic diversity between cultivars and their response to environmental conditions.

The current study aimed to estimate the genetic dimension between eight genotypes of the flax crop using the RAPD technique and to determine the degree of kinship between these structures using the cluster analysis method.

Materials and methods

1- Genotypes: Eight genotypes of flax were used in the study (Sakha 1, Sakha 2, Sakha 3, Sakha 5, Sakha 6, Poloni, Geza and Syrian), which were planted in the fields of the College of Science / University of Kirkuk during the 2020-2021 season according to the RCBD design with three replications.

2- DNA extraction: DNA extraction was carried out in the laboratory of Wahaj Aldna co. in Baghdad city, during the year 2021 according to the attached method (Geneaid@genomic DNA mini kit Plant)) with the extraction kit, which includes the contents listed in Table (1), as the devices and materials mentioned in Table (2) were used in the study):

Table (1): Contents of the extraction kit.

Name	Name
Elution Buffer	**GP1 Buffer**
RNase(10mgml)	**GPX1 Buffer**
Filter Column	**GP2 Buffer**
GD Column	**GP3 Buffer**
2ml collection tube	**W1 Buffer**
	Wash Buffer

Table (2): Materials and devices used in the study.

No	Apparatus	Origin	Company
1	AURA TM PCR Cabinet	Italy	
2	Microspin 12, High-speed Mini-centrifuge	Germany	Bio San
3	V-1 plus, Personal Vortexfor tubes	Germany	Digsystem
4	Bio TDB-100, Dry block thermostatbuilt	Germany	Bio San
5	Biopette VariableVolume 2-20 ul	Germany	
6	Mini-Power Supply 300V,2200V	Chain	
7	Multi Gene Opti Max Gradient Thermal Cycler	USA	Labnet
8	Electrophoreses	USA	CBS, Scientific
9	Document system	USA	Labnet
10	UV.transmission	Farance	Vilber lourmat
11	Microspin	Lativa	Biosan
12	Combi-spin	Lative	Biosan
13	Balance	Germany	Kernpfb
14	Incubation	China	Jrad
15	Microwave	China	Gosonic
16	Water distilater	China	

3- Steps to obtain pure Genomic DNA

Step 1 Tissue dissociation

• Was Took 50 mg of fresh plant leaves.

• The sample was ground to a fine powder and then transferred to a 1.5 ml centrifuge tube.

Step 2 Lysis

• four hundred µl of GP1 Buffer and 5 µl of RNase A were added to the sample tube and mixed with a mixer.

• Samples were incubated at 60°C for 10 minutes. During incubation the tube was inverted every 5 minutes.

• hundred µl of GP2 Buffer was added, mixed with a mixer, and incubated by freezing for 3 minutes.

• Place the filtrate into a 2ml collection tube and transfer the mixture to the filtrate.

• Put the tube in a centrifuge for one minute at 1000 cycles and then remove the filtrate.

• The supernatant was carefully transferred from the 2 mL collection tube to a new 1.5 mL microcentrifuge tube.

Step 3 DNA restriction

• Add one and a half volumes of GP3 Buffer to the solution and mix immediately for 5 seconds.

• Put Genomic DNA (GD) into a 2 ml collection tube.

• Seven hundred µl of the mixture (and any remaining sediment) were transferred to the GD column.

• The samples were placed in a centrifuge at a rate of 14-16000 cycles for two minutes.

• The filtrate was discarded and the GD column was placed back into a 2 ml collection tube.

The remaining mixture was added to the GD column and then centrifuged at 14-16000 cycles for 2 minutes.

The filtrate was discarded and the GD column was placed back into the 2 ml collection tube.

Step 4 Wash the DNA

• Four hundred µL of W1 Buffer was added to the GD column and then centrifuged at 14-16,000 cycles for 30 seconds.

• The filtrate was discarded and the GD column was placed back into a 2 ml collection tube.

• Six hundred µL of Wash Buffer was added to the GD column.

• Centrifuge at 14-16000 cycles for 30 seconds.

• The filtrate was discarded and the GD column was placed back into the 2 ml collection tube.

• Centrifuge for 3 minutes at 14-16000 cycles to dry the column.

Step 5 DNA Elution

• The sample was rinsed in a volume of 100 µl, and the DNA elution step was repeated a second time to increase the DNA recovery and the total elution volume to approximately 200 µl.

• Transfer the dried GD column to a 1.5 mL microcentrifuge cleaning tube.

• hundred µL of preheated elution solution was added to the medium of the GD column (the required elution buffer (200 µL per sample) was preheated to 60 °C simultaneously with the samples being incubated during the second step of the analysis).

• Leave for 3-5 minutes to ensure that the rinse solution is completely absorbed.

• Put in a centrifuge at 14-16000 cycles for 30 seconds to extract purified DNA.

4- DNA Electrophoresis on Agarose Gel

Electrophoresis was performed to determine the DNA segments after the extraction process or to detect the PCR result while in the presence of standard DNA to differentiate the package size of the PCR result on the agarose gel.

5- Primers used in the reaction

Thirteen Primers were used in the study, which are shown in Table (3). Primers were lyophilized, dissolved in free ddH_2O to give a final concentration of 100 µL/µL as stock solution and kept at -20 °C to prepare a concentration of 10 µL/µL as suspension primer, 10 µL of stock solution in 90 µL of ddH_2O free water to reach volume Final 100 µl, checked by IDT (Integrated DNA Technologies, Canada). Table (4) shows the optimum conditions for gene detection.

Table (3): The list of RAPD primer.

	Primer	Sequence
1	OP-I02	GGAGGAGAGG
2	OP-R06	GTCTACGGCA
3	OP-V02	AGTCACTCCC
4	OP-V09	TGTACCCGTC
5	OP-R12	ACAGGTGCGT
6	OP-M14	AGGGTCGTTC
7	OP-T20	GACCAATGCC
8	OP-M20	AGGTCTTGGG
9	OP-V19	GGGTGTGCAG
10	OP-N16	AAGCGACCTG
11	Op-P04	GTGTCTCAGG
12	OP-M05	GGGAACGTGT
13	OP-L05	ACGCAGGCAC

Table (4): Optimum conditions for gene detection.

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min.	40 cycle
2-	Denaturation -2	95°C	1min	
3-	Annealing	35°C	1min	

4-	Extension-1	72°C	1 min	
5-	Extension -2	72°C	10 min.	

5- Determination of the molecular sizes of the pieces

To calculate the molecular size of photo capt detection, the DNA Ladder program (Cerasela et al., 2011) was used and compared to the size of the thermal PCR index.

6- Analyze the results

The results of the RAPD that appeared in the gel were analyzed after converting the descriptive results into numerical data by placing 1 when the band was present and 0 when it was absent on the agarose gel, the data is arranged in a tabular form that includes the results of all the primer for the studied samples. The genetic dimension coefficient between the samples was calculated according to Nies' (Nie and Lie, 1979) using the coefficient of 72, the following equation:

G.D.=1-((2*Nxy)/(Ny+Nx))

Since:

G.D. = the genetic dimension

Nxy = number of bands shared between the two models

X and y represent the two samples

Nx = the number of total bands in the sample x

Ny = The number of total bands in the sample y

The percentage of formal polymorphism of the primer was calculated using the following equation:

$$\text{Polymorphism percentage per Primer} = \frac{\text{number of dissimilar bands in the Primer}}{\text{Total number of Primer bands}} \times 100$$

The percentage of discriminatory ability for each primer was calculated as follows:

$$\%\text{ of discriminant power of each primer} = \frac{\text{no. of dissimilar bands in the primer}}{\text{No. of dissimilar bands for all primer}} \times 100$$

As for the initiator efficiency percentage, it was calculated from the following equation:

$$\text{Efficiency percentage of each primer} = \frac{\text{total number of primer bands}}{\text{Total number of bands for all primers}} \times 100$$

The cluster analysis scheme was drawn according to the UPGMA method (Sneath and Sokal, 1973), using the ready-made program Numerical Taxonomy System (NTSYS-pc) to obtain a dendrogram or genetic dimension.

Results and discussion

genomic DNA extraction

The extracted DNA was good, as a quantity of DNA estimated at 65-90 µg/g was obtained from the leaves of each flax variety included in the study, with a purity ranging between 1.5-1.85. The DNA samples were diluted to obtain a concentration of 50 ng per microliter, which is Appropriate concentration for PCR reactions. Figure 1 shows the DNA samples of the flax cultivars included in the study.

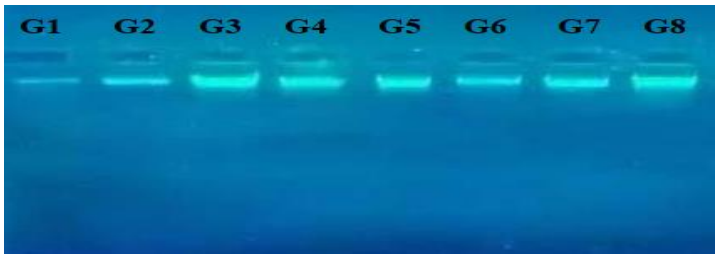


Figure 1: Gel electrophoresis to extract genomic DNA from the eight samples of the flax cultivars under study, on 1% agarose gel, at 5 volume/cm for 30 hours. G1= Sakha2, G2= Sakha3, G3= Giza, G4= Syrian, G5= Sakha1, G6= Poloni, G7= Sakha6, G8= Sakha5.

Loomis (1974) points out that there are many methods for isolating nucleic acids from plants; Because plants are diverse and contain different amounts of nucleic acids, one method for isolating nucleic acids is not suitable for all plants. In extracting the genomic DNA from the flax varieties under study, it was based on the method (Benito et al., 1993), as it is one of the efficient and appropriate methods for isolating the genomic DNA from many plants, including flax, in addition to being an easy and inexpensive method that includes several steps, some of which are mechanical and others chemical, and One of these steps removes a component of the cell without affecting the DNA obtained at the end of the extraction (Farhan and Hammadi, 2016).

Results of RAPD reactions

Thirteen primers were tested and the results of using these primers in RAPD reactions showed a difference in the number of replicate sites and their molecular sizes according to the primer used, resulting from the difference in the number of complement sites for that primer in the genome of each flax cultivar included in this study. (Williams et al., 1990).

The data were analyzed based on the presence or absence of duplicative DNA sites, the sum of binding sites, the sum of packages, the percentage of total packages, as well as the difference in the sizes and numbers of sites, the highest and lowest number of binding sites in each variety and in each primer, and the presence of unique sites and absent sites.

The thirteen primers used in this technique all showed a doubling in locations as shown in the tables (5 and 6) and figures (2-14), as they showed bundles of varying numbers and locations among the varieties under study, and produced a total of 202 bundles, including 192 different bundles with a percentage of 95.05%, while the identical bundles amounted to (Main packages) 10 packages only at a percentage of 4.95%. The magnitude of genetic variance represents the ratio of the differing bundles to the total number of loci (Ahmad, 1999).

The results were divided according to the primers and they appeared as in the table (5), which showed that the primers OP-M20, OP-M05 and OP-L05 showed the highest number of packets compared to the other

primers, which reached 21 bundles for each, while the OP-R06 registered the lowest number of bundles Which reached only 6 bundles, and the superior primers in the number of bundles (OP-M20, OP-M05 and OP-L05) achieved the highest rates for the differentiated bundles (21, 20 and 21 bundles for each of them, respectively), and for the percentages of primers efficiency (10.40% for each of them) and capacity discriminatory for the primer (10.94, 10.42 and 10.94% for each of them, respectively), as well as achieving high rates for the percentage of formal polymorphism for the primer (100, 95.24 and 100% for each of them, respectively), while the primer OP-R06 achieved the lowest rates for the studied indicators it reached 4 bundles, 66.67%, 2.97%, and 2.08% for the number of varying bundles, the formal plurality of the primer, the primer's efficiency and the discriminatory ability of the primer, respectively.

Table (5) shows the outputs of the primers from the total and differentiated packages with their efficiency ratios and discriminatory ability for the studied samples.

Primers name	Total no. of packages	No. of dissimilar packages	% of the polymorphism of the primer	% of primer efficiency	% of the primer's discriminatory power
OP-I02	14	10	71.43	6.93	5.21
OP-R06	6	4	66.67	2.97	2.08
OP-V02	8	7	87.5	3.96	3.65
OP-V09	14	14	100	6.93	7.29
OP-R12	14	14	100	6.93	7.29
OP-M14	14	14	100	6.93	7.29
OP-T20	16	15	93.75	7.92	7.81
OP-M20	21	21	100	10.40	10.94
OP-V19	19	19	100	9.41	9.90
OP-N16	16	16	100	7.92	8.33
Op-P04	18	17	94.44	8.91	8.85
OP-M05	21	20	95.24	10.40	10.42
OP-L05	21	21	100	10.40	10.94
	202	192			

From the results of the table (6) shows that the highest molecular size was 8000 bp for OP-N16 primer, while the lowest molecular size was 100 bp for OP-I02, OP-M20 and OP-V19 scored the highest number of unique sites (4 for each of them), while no unique sites appeared in the OP-102, OP-R06 and OP-V02 primers, and the primers OP-102, OP-V02, OP-M14, and OP-N16 had one location in which packets were absent for each of them, while the other primers did not register absent packets.

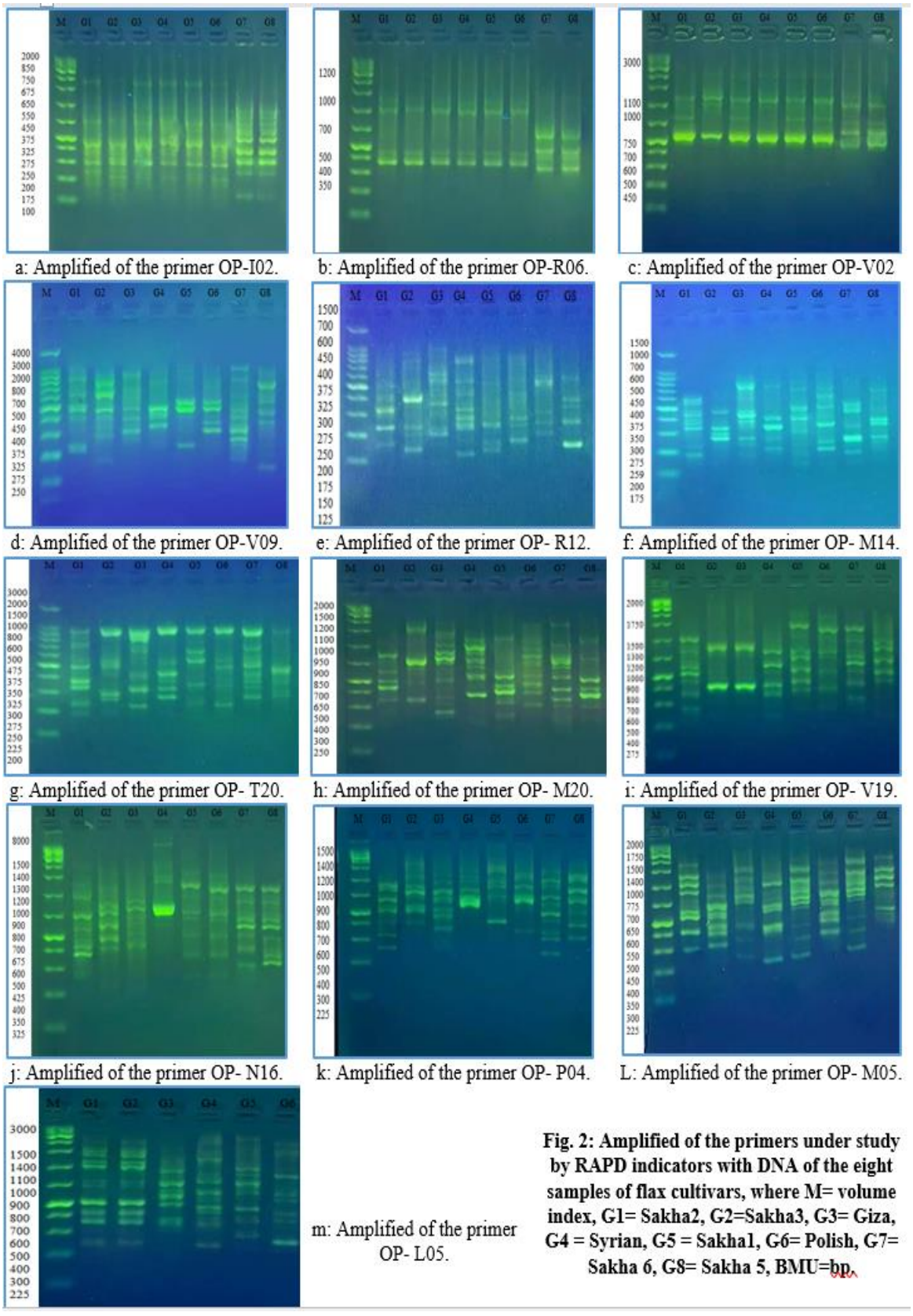
Fu (2005) pooled flaxseed genotypes into 12 major regions and showed that the proportion of RAPD variance was 8.2%, the majority (84.2%) of the RAPD variance was within the accessions of each country and only 15.8% of the variance was present between the accessions from different countries, the results showed that the percentage of polymorphism was 13% in the genotypes of flaxseed. Fu et al. (2002a) also obtained 27.8% RAPD variance in 54 flaxseed genotypes from North American. Fu et al (2001) used random amplified DNA (RAPD) markers to assess genetic diversity

and relationships in 22 Canadian cultivars, 29 selected world cultivars, and 10 strains of L. usitatissimum L. flaxseeds were generally low variance in RAPD and greater variance was detected between Canadian cultivars than the genotypes examined for flaxseed. Despite the high heritability of most traits, the influence of environment was significant in most cases (Diederichsen, 2007).

Ijaz et al (2013) used 120 RAPD primers to check for polymorphisms among linseed cultivars, among these primers 80 were found useful revealing a high degree of similarity of 87%. Gulla et al. (2021) studied DNA polymorphisms using molecular markers in an attempt to characterize five introduced cultivars of flax, and used six RAPD primers belonging to the Operon© series of OPG, OPI, OPM and OPO for preliminary screening for the amplification of gDNAs from these five genotypes amplified primers out of six, It gave packages for all genotypes, of which only one, OPG-02, was found to be polymorphic and reproducible; While the other four primers were less consistent, the mean percentage of similarity was 63% and the mean percentage of polymorphisms observed with RAPD primers (OPG-02, OPG-05, and OPI-02) was 82.2%.

Table (6): Molecular sizes of primers and types of bundles

Primers name	sequence	Molecular sizes	No. of unique packages	No. of absent packages	No. of public packages
OP-I02	GGAGGAGAGG	100-2000	-	1	4
OP-R06	GTCTACGGCA	350-1200	-	-	2
OP-V02	AGTCACTCCC	450-3000	-	1	1
OP-V09	TGTACCCGTC	150-4000	3	-	-
OP-R12	ACAGGTGCGT	125-1500	3	-	-
OP-M14	AGGGTCGTTC	175-1500	1	1	-
OP-T20	GACCAATGCC	200-3000	1	-	1
OP-M20	AGGTCTTGGG	250-2000	4	-	-
OP-V19	GGGTGTGCAG	275-2000	4	-	-
OP-N16	AAGCGACCTG	325-8000	1	1	-
Op-P04	GTGTCTCAGG	225-1500	3	-	1
OP-M05	GGGAACGTGT	225-2000	3	-	1
OP-L05	ACGCAGGCAC	225-3000	2	-	-



a: Amplified of the primer OP-I02. b: Amplified of the primer OP-R06. c: Amplified of the primer OP-V02

d: Amplified of the primer OP-V09. e: Amplified of the primer OP- R12. f: Amplified of the primer OP- M14.

g: Amplified of the primer OP- T20. h: Amplified of the primer OP- M20. i: Amplified of the primer OP- V19.

j: Amplified of the primer OP- N16. k: Amplified of the primer OP- P04. L: Amplified of the primer OP- M05.

m: Amplified of the primer OP- L05.

Fig. 2: Amplified of the primers under study by RAPD indicators with DNA of the eight samples of flax cultivars, where M= volume index, G1= Sakha2, G2=Sakha3, G3= Giza, G4 = Syrian, G5 = Sakha1, G6= Polish, G7= Sakha 6, G8= Sakha 5, BMU=bp.

Estimation of the genetic dimension between varieties based on the results of RAPD

The results of genetic analysis using the RAPD method were used to estimate the genetic distance between the studied cultivars of the flax crop using the genetic program (NTSYS-pc), which depends on the presence of common bundles between the cultivars, based in its analyzes on the Nei equation (Nei and Li, 1979).

The table (7) shows the genetic dimensions among the eight varieties under study, which ranged between (0.365 between the two cultivars Sakha 5 and Sakha 6) and (0.582 between the two cultivars Sakha 1 and Sakha 6). In the event that the genetic material is identical between two cultivars, this indicates that the genetic dimension Between them, it must be equal to zero, and the lowest genetic similarity is equal to 1 (100%) (Esselman et al., 2000), and congruence occurs when there is no genetic variance between varieties using a number of primers, but when using more than one primer or using an indicator other genetics the result may differ due to different regions of association depending on the primer sequence used (Farhan and Hammadi, 2016).

The lowest value that was recorded for the genetic dimension among the studied varieties, which amounted to 0.365 between the two cultivars Sakha 5 and Sakha 6, indicates that they have the largest percentage of similarity in genetic material based on the primers used in the study, while the highest genetic dimension was between the two cultivars Sakha 1 and Sakha 6 with a value of 0.582, this indicates the presence of the least similarity in the genetic material between these two cultivars because they shared the least number of bundles relative to other cultivars, and this difference may be due to their great difference in phenotypic traits (Al-Sahoki, 1990).

Table (7): Results of the genetic dimension among the flax cultivars under study

Varieties	Sakha1	Sakha2	Sakha3	Sakha5	Sakha6	Poloni,	Geza	Syrian
Sakha1	0							
Sakha2	0.451	0						
Sakha3	0.489	0.412	0					
Sakha5	0.492	0.460	0.529	0				
Sakha6	0.582	0.481	0.487	0.365	0			
Poloni	0.380	0.435	0.461	0.445	0.578	0		
Geza	0.393	0.515	0.438	0.424	0.505	0.418	0	
Syrian	0.448	0.543	0.474	0.521	0.531	0.441	0.462	0

The results of the genetic dimension among the eight varieties under study have been invested in finding the genetic relationship that links all these varieties in groups using the cluster analysis method and as shown in Figure (3), which shows that the studied varieties have been divided into three main groups:

The first main group: This group included the two cultivars Sakha 5 and Sakha 6, which were not associated with other cultivars except with a small number of RAPD sites, and they showed uniqueness in the number and length of these sites, which made them separate from everyone in the analysis tree, as they gave a distinct pattern that differs from the groups Other varieties, and this may be attributed to the few genetic changes that occurred to them compared to the rest of the varieties (Nei and Li, 1979).

The second main group: This group also included two cultivars, it is Sakha 2 and Sakha 3, which differed from the groups of other cultivars, which may be attributed to the difference in phenotypic or quantitative characteristics (Nei and Li, 1979).

The third main group: This group included three secondary groups, the first secondary group included the cultivar Syrian, the second secondary group included the cultivar Giza, while the third secondary group included the two cultivars Sakha 1 and Boloni, which ranked second in the degree of genetic dendrogram after the two cultivars Sakha 5 and Sakha 6, an average of 0.380 for the genetic dimension.

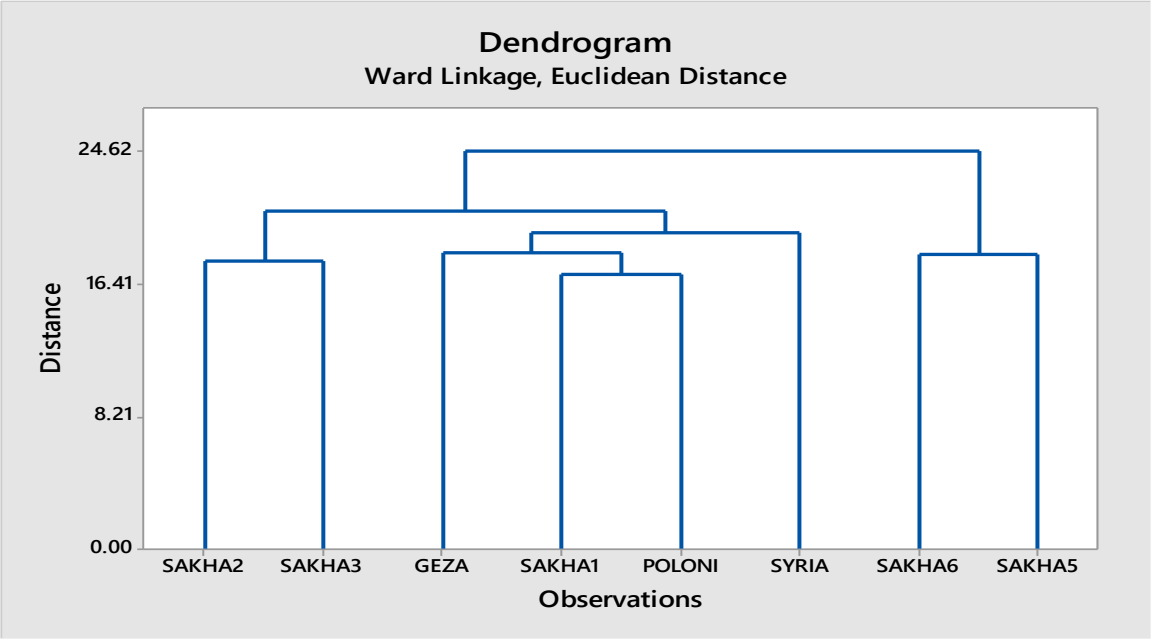


Figure (3): Genetic dendrogram among the eight cultivars under study

One of the reasons for the discrepancy between flax varieties or one of the varieties of other plant species may be the substitution of a nucleotide at one or both of the primer binding sites, which leads to the presence or absence of variation or causes a change in the size of the doubled piece by adding or deleting a small piece of DNA, which It leads to a change in the size of the duplicating segments, as deleting one of the two primers binding sites results in either a segment loss or an increase in the size of the duplicative segment, or as a result of inserting a large DNA segment between the primer binding sites, which exceeds the ability of PCR and the result is a loss of segments (Weising et al. 1995), and therefore the outputs of those primers showed different bundles in location, number and size, and among these bundles there are what are known as unique bundles that are important in determining the genetic fingerprint of a particular variety without the other items included in the study, as the presence of unique packages reduces cost and effort, because they give high discriminatory results in a record time, as well as the case in the absence of a bundle of a particular variety that is also important in determining the genetic fingerprint of that variety among the studied varieties, and the primers that produce several different bundles have a greater chance of finding unique bundles of varieties, with opportunities remaining for the primers that produce a small number of different bundles, while the general bundles indicate the presence of a common piece of DNA in those varieties and rely on these

bundles to identify the patterns of unknown origin or missing classification, because they often represent a site it is shared by all members of the same genus or species (Williams et al., 1993).

Fu et al. (2002b) analyzed seven flaxseed species in the genus Linum using 29 RAPD culture primers, and cluster analyzes based on similarity estimates showed that the fiber cultivars were more closely related (or similar to each other) and classified as a homogeneous group.

Ijaz et al. (2013) showed that the results of the cluster analysis grouped the genotypes into five main groups, A, B, C, D and E where group E was the largest group of the tree. Comparing each strain individually with the rest of the genotypes showed a similarity of 90 to 84%. Strains L-7, L-12 and L-31 appeared as the most diverse genotypes when compared with all other genotypes, with a mean difference of 16%. Adugna and Labuschagne (2003) also reported that cluster analysis classified the genotypes into five categories according to their original sources, and the characteristics of the number of days to flowering, maturity and oil content played a major role in distinguishing the genotypes. Tadesse et al (2009) showed that the maximum distance observed between the first and fourth groups (D2 = 762.3), and between the first and third groups (880.65), while the lowest observed between the eighth and ninth groups was 17.4 and between the ninth and tenth groups, which was 20,837.

Gulla et al. (2021) reported that the resulting dendrogram from flaxseed genotypes showed two main groups, the first group consists of cultivars NL-356, RLC-156, Padmini and LCK-1625, and the second group consists of cultivar LCK-2627, these two groups were joined together at the genetic distance level of 0.43, and the first group was divided again into subgroup 1 (NL- 356, RLC-156 and Padmi) and subgroup 2 (LCK-1625) were included at 0.92 and 0.66 genotypic distances, respectively.

Results of major component assignment of genotypes based on RAPD indicators

The results of the Principal Analysis Component (PCA) shown in Figure (4) showed a clear match with the results of the genetic dendrogram shown in Figure (3). The first group included Sakha 5 and Sakha 6, while the second group included Sakha 2 and Sakha 3, while the third main group included the rest of the varieties.

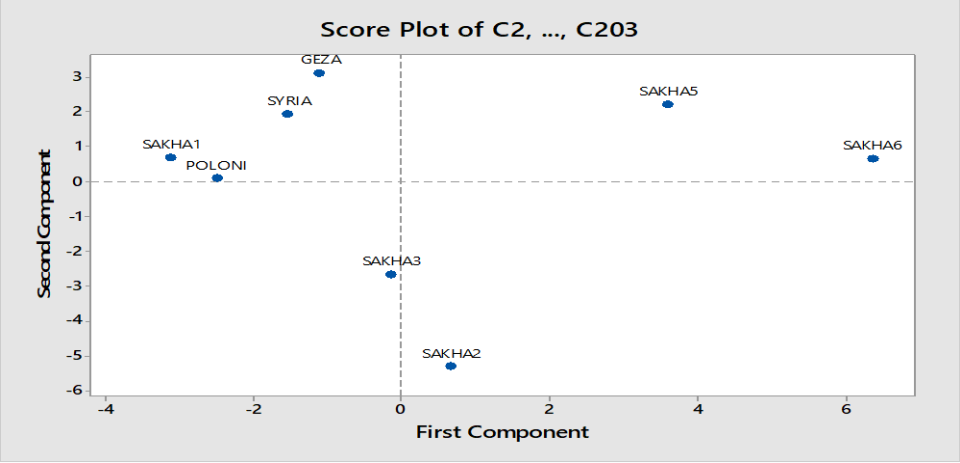


Figure (4): Principal Analysis Component

References

Adugna, W. and M.T. Labuschagne, 2003. Cluster and canonical variate analyses in multilocation trials of linseed. J. Agric. Sci., 140(3): 297-304.

Ahmad, F. (1999). Random Amplified Polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual Cicer species. Theor. Appl. Genet. 98 : 657-663.

Akbar, M., Mahmood, T., Anwar, M., Ali, M., Shafiq, M. and Salim, J. (2003). Linseed improvement through genetic variability, correlation and path coefficient anal-ysis. Inter. J. Agri. Biol., 5: 303-305.

Al-Sahoki, Medhat Majid. (1990). Yellow corn, its production and improvement. Higher Education Press, Baghdad.

Bakry, A.B., Omar, M.I., Tarek, A.E.E., & Mohamed, F.E. (2014). Performance assessment of some flax (Linum usitatissimum L.) varieties using cluster analysis under sandy soil conditions. Agricultural Sciences, 5, 677-686.

Begum, H., A.K.M.M. Alam, M.J.A. Chowdhury, M.I. Hossain. (2007) Genetic divergence in linseed (Linum usitatissimum L.). Int. J. Sustain. Crop. Prod., 2(1): 04-06.

Benito, C; Figueiras, A. M.; Zaragozal, C.; Gallego, F. j. and Dela Penal A. (1993). Rapid identification of Triticeae geno types from single seed using the polymerase chain reaction. Journal Plant Molecular Biology. 21 (1): 181-183.

Cerasela, P., Lazar, A., Irina, P., Maria, I., Giancarla, V. and Banu C. (2011). Somaclonal variation at the nucleotide sequence level revealed by RAPD and ISSR markers. J. of hortic., Fores. and Biotech., 15(4): 119-123.

Diederichsen, A. (2007). Ex situ collections of cultivated flax (Linum usitatissimum L.) and other species of the genus Linum L. Genet. Res. and Crop Evol., 54(3): 661-678.

Farhan, M. A., & Hamadi, A. M. A. (2016). Detection of genetic diversity for number of Iraqi maize cultivars (Zea mays L.) by using the RAPD-PCR technique. Journal of university of Anbar for Pure science, 10(3).

Fotokian, M.H., Agahi, K., Ahmadi, J., & Vaezi, B. (2014). Selection of barley advanced lines at rainfed conditions using regression and cluster analyses. International Journal of Biosciences. Vol. 4, No. 6, pp. 80-88. ISSN: 2220-6655.

FU, Y.B., 2005. Geographic patterns of RAPD variation in cultivated flax, Crop Sci., 45: 1084-1091.

Fu, Y.B., A. Diederichsen, K.W. Richards and G. Peterson. (2001). Genetic diversity within a range of cultivars and landraces of flax (Linum usitatissimum L.) as revealed by RAPDs, Genet. Res. Crop. Evo., 49(2): 167-174.

Fu, Y.B., G.G. Rowland, S.D. Duguid and K.W. Richards. (2002a). RAPD Analysis of 54 North American Flax Cultivars. Crop Sci., 43(4): 1510-1515.

Fu, Y.B., G.W. Peterson, A. Diederichsen and K.W. Richards. (2002b). RAPD analysis of genetic relationships of seven flax species in the genus Linum L. Genet. Res. Crop Evol., 49: 253-259.

Gulla, B., Jadhav, V., & Dudhare, M. S. (2021). Assessment of genetic diversity and physiochemical analysis of linseed (Linum usitatissimum) genotypes.

Ijaz, Ambreen; Anum s hahbaz; Ihsan ullah; Sadia Ali; Tayyaba Shaheen; Mahmood-ur-Rehman; Usman Ijaz and Sami Ullah. (2013). Molecualar Characterization of Linseed Germplasm Using RAPD DNA Fingerprinting Markers. American-Eurasian J. Agric. & Environ. Sci., 13 (9): 1266-1274, 2013.

Karp, A., K.V. K resovich, Bhat, W.G. Ayad and T. Hodgkin. (1997). Molecular tools in plant genetic resources conservation: a guide to the technologies. In: IPGRI Technical Bulletin No.2. International Plant Genetic Resources Institute, Rome, Italy.

Loomis, M. D. (1974). Overcoming problems of phenolics in the isolation of plant enzyme and organelles. Meth. Enzymol. 31:528-545.

Nei, M. and W. H.Li. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences. 76(10): 5269-5273.

Rameeh, V. (2015). Heritability, genetic variability and correlation analysis of some important agronomic traits in rapeseed advanced lines. Cercetări Agronomice în Moldova Vol. XLVIII, No. 4 (164). DOI:10.1515/cerce-2015-0054.

Saunders, J.A. and Mischke, S. Hemeida, (2001). The use of AFLP technique for DNA fingerprinting in plants. Beckman Coulter, Inc.

Sneath, P.H.A. and Sokal. (1973). Numerical taxonomy – the principals and practice of numerical classification. W.H. Freeman and Co., San Francisco.

Tadesse, T., H. Singh and B. Weyessa, (2009). Genetic Divergence in Linseed Germplasm. J. Innov. Dev. Strategy. 3(2): 13-20.

Weising, K.; Atkinson, R.G. and Gardner, R. C. (1995). Genomic fingerprinting by microsatellite-primed PCR: a critical evaluation, PCR Methods

Williams, J. G.; Kubelik, A. R.; Livak, K. J.; Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531–6535.

Williams, J. G.; Reiter, R. S.; Young, R. M. and Scolhik, P. A. (1993). Genetic mapping of mutations using phenotypic pools and mapped RAPD markers. Nucleic Acid Research. 21: 75-95.